

10/816, 720
EXHIBIT 2

<http://genomobiology.com/2001/3/1/reviews/3001.1>

Protein family review
The Wnts
Jeffrey R Miller

Address: Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN 55455, USA.
E-mail: miller380@mail.med.umn.edu

Published: 28 December 2001

Genome Biology 2001, 3(1):reviews3001.1-3001.15

The electronic version of this article is the complete one and can be found online at <http://genomobiology.com/2001/3/1/reviews/3001>

© BioMed Central Ltd (Print ISSN 1465-6906; Online ISSN 1465-6914)

Summary

The *Wnt* genes encode a large family of secreted protein growth factors that have been identified in animals from hydra to humans. In humans, 19 *WNT* proteins have been identified that share 27% to 83% amino-acid sequence identity and a conserved pattern of 23 or 24 cysteine residues. *Wnt* genes are highly conserved between vertebrate species sharing overall sequence identity and gene structure, and are slightly less conserved between vertebrates and invertebrates. During development, *Wnts* have diverse roles in governing cell fate, proliferation, migration, polarity, and death. In adults, *Wnts* function in homeostasis, and inappropriate activation of the *Wnt* pathway is implicated in a variety of cancers.

Gene organization and evolutionary history

Gene organization

In humans, 19 *WNT* genes have been identified and the chromosomal locations of each is known (see Table 1) [1-6]. Several human *WNT* genes are located very close to each other in the genome [7,8]; these include *WNT6* and *WNT10a*, which are located immediately adjacent to one another on chromosome 2 (about 6.4 kilobases (kb) apart), and *WNT1* and *WNT10b*, which are located adjacent to each other on chromosome 12 (about 8.1 kb apart). *WNT6* and *WNT10a* are transcribed in opposite directions, whereas *WNT1* and *WNT10b* are expressed from the same strand of DNA. Several additional pairs of *WNT* genes are also clustered within the human genome, including *WNT2* and *WNT16* (about 4 megabases (Mb) apart), *WNT3a* and *WNT14* (about 250 kb apart), and *WNT3* and *WNT15*. In the mouse, there are at least 18 *Wnt* genes and the locations of all but two of them have been determined [1-3,5,6]. As in humans, the mouse *Wnt1/Wnt10b*, *Wnt6/Wnt10a*, and *Wnt3/Wnt15* gene pairs are each located on the same chromosomes, and in the case of the *Wnt1/Wnt10b* and *Wnt6/Wnt10a* pairs the close proximity of these genes has been conserved from mouse to human. Interestingly, in the *Drosophila* genome, the paralogous genes *wingless (wg)*, *DWnt6* and *DWnt10*, are located immediately adjacent to one another on the second chromosome and are all transcribed in the same orientation. Thus, it is

possible that there was an ancient cluster of *Wnt* genes consisting of *Wnt1*, *Wnt6* and *Wnt10* in a common ancestor of vertebrates and arthropods. In vertebrates, this cluster may have been duplicated with subsequent loss of *Wnt1* from one cluster and *Wnt6* from the other.

The majority of human *WNT* genes contain four coding exons, with exon 1 containing the initiation methionine (Figure 1a) [8]. *WNT* genes that differ from this pattern include *WNT14*, with three exons, *WNT2*, *WNT5b*, and *WNT11*, with five exons, and *WNT8b* with six exons. Several *WNTs* - *WNT2b/13*, *WNT8a/d*, and *WNT16* - have alternative amino or carboxyl termini, which result from the use of alternative 5' or 3' exons.

Evolutionary history

The deduced evolutionary relationships of 18 of the 19 known human *WNT* genes are shown in Figure 2. The majority of *Wnt* proteins share about 35% amino-acid sequence identity, although members of a subgroup (those with the same numeral, such as *WNT3* and *WNT3a*) share increased sequence identity (from 58% to 83%) and some overlapping sites of expression. Members of subgroups are not closely linked within the genome, however, suggesting that they were generated by gene-translocation or genome-duplication events, not by local duplication events.

Table 1

Chromosomal locations of WNT genes in human and mouse

Human		Mouse		References	Accession numbers [†]	
Gene	Location	Gene	Location*		Human	Mouse
WNT1	12q13	Wnt1	15	[87-91]	X03072	K02593
WNT2	7q31	Wnt2	6 (4.2 cM)	[92,93]	X07876	AK012093
WNT2b/13	1p13	Wnt2b/13	3 (49.0 cM)	[94-96]	XM052111, XM052112	AF070988
WNT3	17q21	Wnt3	11 (63.0 cM)	[97-100]	AY009397	M32502
WNT3a	1q42.13	Wnt3a	11 (32.0 cM)	[101-103]	AB060284	X56842
WNT4	1p35	Wnt4	4	[100,104]	AY009398	M89797
WNT5a	3p14-p21	Wnt5a	14 (14.8 cM)	[104-106]	L20861	M89798
WNT5b	12p13.3	Wnt5b	6 (56.2 cM)	[104,107]	AB060966	M89799
WNT6	2q35	Wnt6	1	[104,108,109]	AY009401	M89800
WNT7a	3p25	Wnt7a	6 (39.5 cM)	[104,106,110,111]	D83175	M89801
WNT7b	22q13.3	Wnt7b	15 (46.9 cM)	[100,104,112,113]	AB062766	M89802
WNT8a/d	5q31	Wnt8a		[114,115]	AB057725, AY009402	Z68889
WNT8b	10q24	Wnt8b	19 (43.0 cM)	[116-118]	Y11094	AF130349
WNT10a	2q35	Wnt10a	1	[109,119]	AB059569	U61969
WNT10b/12	12q13.1	Wnt10b	15 (56.8 cM)	[106,119-124]	U81787	U61970
WNT11	11q13.5	Wnt11	7	[106,125]	Y12692	X70800
WNT14	1q42	-		[103,126]	AB060283	
WNT15	17q21	Wnt15	11	[126]	AF028703	AF031169
WNT16	7q31	Wnt16		[127,128]	XM031374, XM004884	AF172064

*Locations of mouse genes give the chromosome and the distance in centimorgans (cM) from the telomere. †Accession numbers are for GenBank [3].

Wnt genes have been identified in vertebrates and invertebrates, but appear to be absent from plants, unicellular eukaryotes such as *Saccharomyces cerevisiae* and from prokaryotes. To date, in vertebrates, 16 Wnt genes have been identified in *Xenopus*, 11 in chick, and 12 in zebrafish [5]; in invertebrates, *Drosophila* has seven Wnt genes, *Caenorhabditis elegans* five and *Hydra* at least one [5]. The apparent evolutionary relationships between selected invertebrate and vertebrate Wnt genes are shown in Figure 2b. In vertebrates, the orthologs in different species are highly similar in sequence. For example, human WNT1 and mouse Wnt1 are 98% identical, and human WNT5a and *Xenopus* Wnt5a are 84% identical at the amino-acid level. Phylogenetic analyses of vertebrate and invertebrate Wnts demonstrate orthologous relationships between several human and *Drosophila* Wnts (Figure 2b). The sequence identity between orthologous proteins in humans and flies ranges from 21% between human WNT8a/d and *Drosophila* DWnt8 to 42% sequence identity between human WNT1 and *Drosophila* Wingless (Wg). The evolutionary relationship between the five *C. elegans* Wnt genes and human WNT genes is less apparent, making it

difficult to determine which *C. elegans* Wnt genes may have orthologs in the human genome.

Characteristic structural features

Human WNT proteins are all very similar in size, ranging in molecular weight from 39 kDa (WNT7a) to 46 kDa (WNT10a) [3]. *Drosophila* Wnt proteins are also similar to this, with the exception of Wg, which is approximately 54 kDa and has an internal insert not found in vertebrate Wnts, and DWnt3/5, which is about 112 kDa [3]. Very little is known about the structure of Wnt proteins, as they are notoriously insoluble, but all have 23 or 24 cysteine residues, the spacing of which is highly conserved (Figure 1b), suggesting that Wnt protein folding may depend on the formation of multiple intramolecular disulfide bonds. Analysis of the signaling activities of chimeric Wnt proteins has shown that the carboxy-terminal region of Wnt proteins may play a role in determining the specificity of responses to different Wnts [9]. Furthermore, deletion mutants lacking the carboxy-terminal third of a Wnt protein can act as

<http://genomebiology.com/2001/3/1/reviews/3001.3>

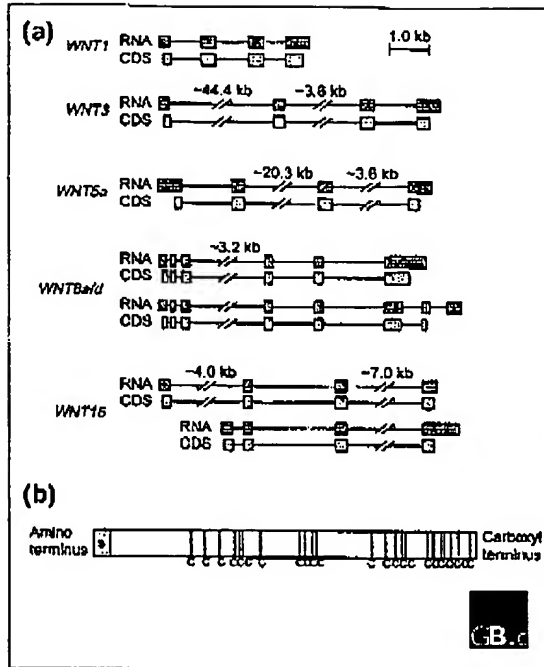


Figure 1
(a) Structures of selected members of the human WNT gene family. Exons are shown as boxes and introns as lines. For each gene, 'RNA' represents the portion of the gene that is transcribed and 'CDS' represents the portion that encodes protein. *WNT8a/d* is an example of a gene with 3' alternative splicing and *WNT16* is an example of a gene with alternatively used 5' exons. (b) Structural features of the Wnt protein. The amino terminus contains a signal sequence (S). All Wnts contain 23 or 24 conserved cysteine residues (C) with similar spacing, suggesting that the folding of Wnt proteins depends on the formation of multiple intramolecular disulfide bonds.

dominant-negatives in a cell-non-autonomous manner [10], suggesting that the amino-terminal region may mediate interactions with Wnt receptors but requires the carboxyl terminus to activate these receptors.

Localization and function

Post-translational modifications and secretion

Wnt proteins have an amino-terminal signal sequence, can act in a cell non-autonomous manner, and are present in the secretory pathway, indicating that they are secreted proteins [11]. In addition, genetic analyses of Wg signaling in *Drosophila* uncovered mutations in the *porcupine* gene that show a lack of Wnt activity due to the retention of Wg protein in the endoplasmic reticulum [12-14]. The *porcupine* gene is predicted to encode a protein with eight transmembrane domains and has a perinuclear localization in transfected

cells [14]; overexpression of *porcupine* does not increase levels of secreted Wg but does change the pattern of Wg glycosylation [14]. In worms, *mom-1* encodes a *porcupine* homolog and, when mutated, phenocopies mutants of *mom-2*, which encodes a Wnt, suggesting that the function of *porcupine* is conserved [15,16]. Although size chromatography suggests that Wg is secreted as a multimer, it remains unclear whether Wnt proteins in general are secreted as monomers, oligomers, or as part of a multi-protein complex [17]. Wnt proteins are glycosylated, but mutation of some or all of the predicted glycosylation sites in mouse *Wnt1* does not abolish its activity in cultured cells [18]; these modifications may thus be unimportant for Wnt function.

Subcellular localization

Once secreted, Wnt proteins associate with glycosaminoglycans in the extracellular matrix and are bound tightly to the cell surface [19,20]. Although Wnts are found in tight association with the plasma membrane, it is possible to collect active Wnt from the medium of cultured cells [21,22]. Beyond this information, the localization of Wnt proteins in vertebrates is poorly understood. Examination of the localization of Wg in *Drosophila*, however, has provided critical insights into the subcellular distribution of Wnt proteins and the importance of this distribution for signaling activity. In the embryonic epidermis, Wg is found inside cells that secrete Wg and in association with the plasma membrane of secreting cells and non-secreting cells several cell diameters from the Wg source [23]. Wg is also prevalent in vesicles and multi-vesicular bodies of non-Wg-producing cells anterior to the source of Wg, suggesting that Wg is endocytosed [23,24]. This idea is supported by examination of *shibire* embryos, which have a mutation in dynamin, a critical component of the endocytic machinery; these mutants have defects in Wg distribution, and Wg signaling activity is compromised [25]. Similarly, expression of a dominant-negative form of *shibire* also reduces Wg activity [26]. Endocytosis may also help to limit the distribution of Wg signal. In contrast to cells anterior to the Wg source, cells posterior to Wg-producing cells have much lower levels of Wg in endocytic vesicles, and this asymmetry in distribution mirrors the observation that Wg acts over a much shorter range towards the posterior than towards the anterior. This difference in Wg distribution appears to be due to rapid degradation of endocytosed Wg in posterior cells [27]. The spatially restricted pattern of Wg degradation is regulated by signals through the epidermal growth factor (EGF) receptor that hasten the destruction of Wg in posterior cells [27].

Association of Wg with specific membrane microdomains also appears to play a role in controlling the distribution of Wg signals during *Drosophila* development. In imaginal discs, Wg is found in specialized membrane vesicles called argosomes, which are thought to be derived from lipid raft microdomains [28]. Incorporation of Wg into argosomes requires heparan sulfate proteoglycans, suggesting that

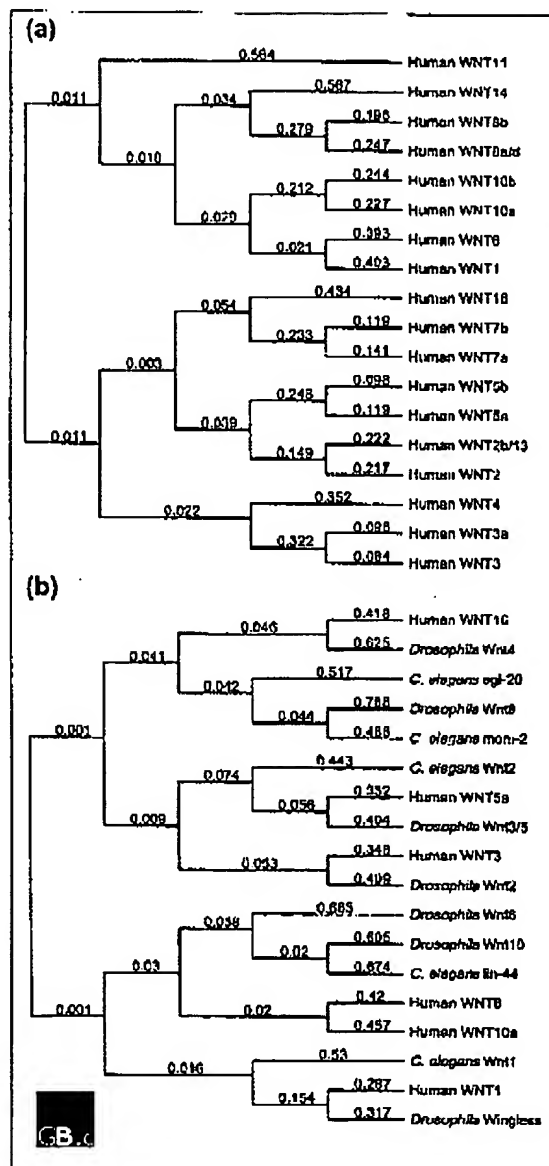


Figure 2
Predicted evolutionary relationships between members of the Wnt gene family. (a) Predicted relationships between 18 of the 19 known human WNT protein sequences; WNT15 was omitted because only a partial sequence is available. (b) Predicted evolutionary relationships between selected human WNT proteins (representing each large grouping shown in (a)) and Wnt proteins from mouse, Xenopus, Drosophila, and *Caenorhabditis elegans*. Sequences were aligned using the ClustalW program; trees were constructed from the alignments using the neighbor-joining method and are diagrammed using midpoint rooting. Numbers indicate branch lengths.

proteoglycans play a role in sorting Wg to specialized membrane microdomains in Wg-producing cells or, alternatively, may play a role localizing Wg in distinct endocytic compartments in receiving cells.

Polarized distribution of *wg* transcripts in embryonic epithelial cells is also required for optimal signaling activity. High-resolution *in situ* hybridization analyses demonstrate that *wg* transcripts are localized apically in the embryonic epidermis and that this distribution is mediated by two *cis*-acting elements found in the 3' UTR of the *wg* mRNA [29]. Mutation of these elements results in uniform localization of *wg* transcripts and impaired Wg protein distribution and signaling. The asymmetric distribution of *wg* transcripts is dependent on dynein-mediated microtubule transport [30].

Function

Wnt and Wnt receptors

Reception and transduction of Wnt signals involves binding of Wnt proteins to members of two distinct families of cell-surface receptors, members of the Frizzled (Fzd) gene family and members of the LDL-receptor-related protein (LRP) family [31,32]. The canonical Fzd receptor has an amino-terminal cysteine-rich domain (CRD) that binds Wnt, seven transmembrane domains and a short cytoplasmic tail containing a consensus PDZ domain binding motif (S/T-X-V in the single-letter amino-acid code) at the carboxyl terminus. The CRD forms a novel protein fold with a conserved dimerization interface that may be important for Wnt binding [33]. Fzd receptors have been identified in vertebrates and invertebrates; there are ten known members in humans and mice, four in flies, and three in worms. The general structure of Fzd receptors resembles that of seven-transmembrane G-protein-coupled receptors, suggesting that Fzd proteins may use heterotrimeric G proteins to transduce Wnt signals. Several recent studies provide evidence consistent with this idea, showing that a subgroup of Fzd receptors can signal through the pertussis-toxin-sensitive subclass of heterotrimeric G proteins to stimulate an increase in intracellular Ca^{2+} and activate protein kinase C (PKC) [34-38]. Heterotrimeric G proteins do not appear to be involved in transducing Wnt/Fzd signals that regulate the cytoskeleton-associated protein β -catenin, however (see below).

Two members of the vertebrate LRP family, LRP-5 and LRP-6, can bind Wnts and may form a ternary complex with a Wnt and a Fzd [39]. Mutations in LRP-6 in mice result in developmental defects similar to those seen in mice deficient for several individual Wnt genes [40], and overexpression of LRP in *Xenopus* can activate the Wnt pathway [39]. In *Drosophila*, arrow, the ortholog of LRP5 and LRP6, is required for optimal Wg signaling [41]. Although the mechanism of LRP signaling is unclear, recent evidence suggests that binding of the cytoplasmic domain of LRP to the Wnt antagonist Axin may play a role in Wnt pathway activation [42].

<http://genomebiology.com/2001/3/1/reviews/3001.5>

In addition to the Fzd and LRP receptors, cell-surface proteoglycans also appear to have a role in the reception of Wnt signals. For example, genetic analyses in *Drosophila* have shown that several genes required for optimal Wg signaling encode cell-surface proteoglycans of the glypican family [43,44] and proteins involved in proteoglycan synthesis [45-47]. Furthermore, QSulf1, an avian protein related to heparan-specific *N*-acetyl glucosamine sulfatases, has also been shown to regulate heparan-dependent Wnt signaling in cultured cells [48]. It is unclear at this time how proteoglycans modulate Wnt signaling, but current suggestions include concentrating Wnt proteins at the cell surface or presenting Wnt ligands to cell-surface receptors.

Secreted modulators of Wnt signaling

Wnt signals are modulated extracellularly by diverse secreted proteins, including members of the Frizzled-related protein (FRP or FrzB) family [49], Wnt-inhibitory factor-1 (WIF-1) [50], Cerberus [51], and Dickkopf (Dkk) [52]. FRPs, WIF-1, and Cerberus can bind Wnt proteins directly and are thought to antagonize Wnt function by preventing their interaction with Fzd receptors. FRPs can also interact with Fzds, suggesting that a second way in which FRPs might antagonize Wnt signaling is through the formation of a non-functional complex with Fzd receptors. Humans have at least five FRP genes, and the specificity of each FRP for different Wnts remains to be determined. Dkk does not bind Wnts but instead interacts with the extracellular domain of LRPs, thereby blocking activation of Wnt signaling [42,53,54]. Four Dkk genes have been identified in vertebrates, including *Dkk2*, which does not act as a Wnt antagonist but rather can stimulate Wnt signaling [55].

Intracellular signaling pathways

Wnt signals are transduced through at least three distinct intracellular signaling pathways including the canonical 'Wnt/ β -catenin' pathway, the 'Wnt/ Ca^{2+} ' pathway, and the 'Wnt/polarity' pathway (also called the 'planar polarity'

pathway) [5,56-62]. Distinct sets of Wnt and Fzd ligand-receptor pairs can activate each of these pathways and lead to unique cellular responses. The Wnt/ β -catenin pathway primarily regulates cell fate determination during development, whereas the major function of the Wnt/polarity pathway is regulation of cytoskeletal organization. The biological function of the Wnt/ Ca^{2+} pathway is unclear.

The canonical Wnt/ β -catenin pathway is intensely studied, and on the basis of current literature I propose the model illustrated in Figure 3a [59,63,64]. Signaling through this pathway depends on the levels of β -catenin in the cell. In the absence of Wnt, β -catenin is targeted for degradation by a multi-protein destruction complex. Wnt signaling antagonizes the destruction complex, leading to the accumulation of β -catenin and activation of target genes. Up-to-date lists of proteins involved in Wnt/ β -catenin signaling and the potential roles of each of these proteins can be found on the worldwide web [5,60,62].

The Wnt/ Ca^{2+} pathway involves an increase in intracellular Ca^{2+} and activation of PKC; it can be activated by a distinct group of Wnt ligands and Fzd receptors from those that activate other pathways, including Wnt5a, Wnt11 and Fzd2 (Figure 3b) [58,61,62]. The Wnt/ Ca^{2+} pathway involves activation of a heterotrimeric G protein, an increase in intracellular Ca^{2+} , and activation of calcium/calmodulin-regulated kinase II (CamKII) and PKC [34,35,37]. The downstream targets of CamKII and PKC are currently unknown, but it has been shown that activation of the Wnt/ Ca^{2+} pathway can antagonize the Wnt/ β -catenin pathway in *Xenopus*, although it is unclear at what level this interaction occurs [65].

Wnt/polarity signaling regulates the polarity of cells through regulation of their cytoskeletal organization (Figure 3c) [56,57,62]. In vertebrates, Wnt/polarity signaling is thought to control polarized cell movements during gastrulation and neurulation [66-70]. In *Drosophila*, Wnt/polarity signaling

Figure 3 (see the figure on the next page)

The known Wnt signaling pathways. (a) In the Wnt/ β -catenin pathway, Wnt signaling depends on the steady-state levels of the multi-functional protein β -catenin. In the absence of Wnt signal, a multi-protein destruction complex that includes the adenomatous polyposis coli protein (APC) and a member of the Axin family facilitates the phosphorylation of β -catenin by glycogen synthase kinase 3 (GSK3). GSK3 substrates also include APC and Axin; phosphorylation of each of these proteins leads to enhanced binding of β -catenin. Phosphorylated β -catenin is bound by the F-box protein β -TrCP, a component of an E3 ubiquitin ligase complex, and is ubiquitinated; the ubiquitin tag marks β -catenin for destruction by the proteasome. When a cell is exposed to a Wnt, the Wnt interacts with its coreceptors Frizzled and LRP. Activation of Frizzled and LRP leads to the phosphorylation of Dishevelled (Dsh), a cytoplasmic scaffold protein, perhaps through stimulation of casein kinase Ia (CKIa) and/or casein kinase II (CKII). Dsh then functions through its interaction with Axin to antagonize GSK3, preventing the phosphorylation and ubiquitination of β -catenin. In vertebrates, inhibition of GSK3 may involve the activity of GSK3 binding protein (GBP/Prat), which binds to both Dsh and GSK3 and can promote dissociation of GSK3 from the destruction complex. Unphosphorylated β -catenin escapes degradation, accumulates in the cell, and enters the nucleus, where it interacts with members of the TCF/LEF family of HMG-domain transcription factors to stimulate expression of target genes. In addition to the components of the Wnt/ β -catenin pathway described here, many additional proteins with potential roles in regulating Wnt/ β -catenin signaling have been reported including the phosphatase PP2A and the kinases Akt/protein kinase B, integrin-linked kinase (ILK), and PKC. (b) Signaling through the Wnt/ Ca^{2+} pathway appears to involve activation of the two pertussis-toxin-sensitive G proteins, $\text{G}_{\alpha o}$ and $\text{G}_{\alpha i}$, in combination with $\text{G}_{\alpha q}$ [34,35]. G-protein activation then leads to an increase in intracellular Ca^{2+} and the subsequent stimulation of Ca^{2+} /calmodulin-dependent kinase II (CamKII) [37]. Activation of the Wnt/ Ca^{2+} pathway also results in stimulation of PKC activity in the form of the translocation of PKC to the plasma membrane [34]. Downstream targets of the Wnt/ Ca^{2+} pathway have not been identified. (c) The Wnt/polarity pathway, which regulates cytoskeletal organization; the *Drosophila* Wnt/polarity pathway that regulates the polarity of trichomes in the wing is shown as an example. In this case, the nature of the polarity signal is not known.

6 Genome Biology Vol 3 No 1 Miller

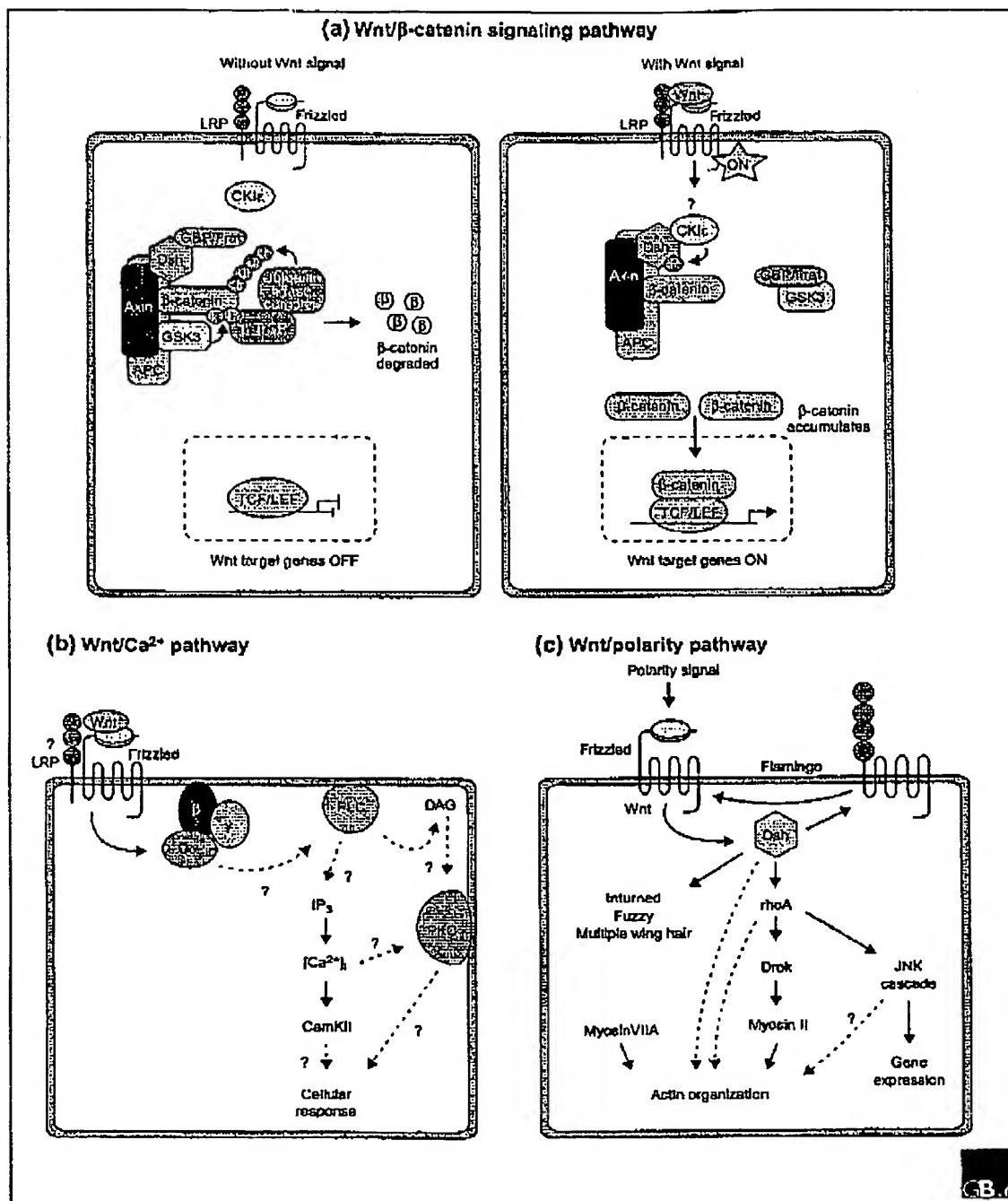


Figure 3 (see the legend on the previous page)

<http://genomebiology.com/2001/3/1/reviews/30017>

is required for the appropriate orientation of trichomes - or hairs - of the adult wing, and for appropriate chirality of ommatidia in the eye, and may regulate asymmetric cell divisions of certain neuroblasts [56,71,72]. The only molecules known to function in both the vertebrate and the invertebrate Wnt/polarity pathways are members of the Fzd family and the cytoplasmic scaffold protein Dsh. The regulation of gastrulation movements in vertebrates also requires the activity of *Wnt11*, which may signal through Fzd7 to regulate protrusive activity during convergent extension [66,67]. In flies, genetic analyses have identified a number of potential components of the Wnt/polarity pathway in addition to Dfzd1 and Dsh, including the small GTPase DrhoA, *Drosophila* rho-associated kinase (Drok), Jun N-terminal kinase (JNK), myosin II, myosin VIIA, and the products of the novel genes *flamingo/starry night*, *fuzzy*, *inturned*, and *strobismus/van gogh* [56,72]. A Wnt ligand for the Wnt/polarity pathway has not been identified in flies, however, and it remains to be seen how much of the intracellular signaling mechanism has been conserved between vertebrates and invertebrates.

Several studies have suggested that distinct classes of Wnts signal through either the Wnt/ β -catenin pathway or the Wnt/ Ca^{2+} pathway [58]; for example, overexpression studies in *Xenopus* have shown that XWnt1, XWnt3a, XWnt8, and XWnt8b can stimulate the Wnt/ β -catenin pathway whereas XWnt4, XWnt5a, and XWnt11 can stimulate the Wnt/ Ca^{2+}

pathway [59]. Furthermore, the separation of Wnts into these two distinct functional classes is mirrored by the classification of Fzd proteins into similar functional groups on the basis of their ability to activate one or other pathway in overexpression assays. Although this classification of Wnts, which partially mirrors their evolutionary relationships, may provide a useful tool for predicting the function of Wnts and Fzds, the relationship between specific Wnts and the intracellular pathway they use is not fixed. For example, overexpression of XWnt5a in combination with human FZD5 in *Xenopus* embryos results in activation of the Wnt/ β -catenin pathway [73], suggesting that the activity of Wnts *in vivo* will be determined by the repertoire of Fzd receptors present at the cell surface.

Important mutants and developmental functions

Loss-of-function mutations in 9 of the 18 mouse Wnt genes have been generated, and the phenotypes of mutant embryos demonstrate the diverse functions of Wnt genes during embryogenesis (Table 2). For example, knocking out *Wnt1* results in a dramatic loss of a portion of the midbrain and deletion of the rostral cerebellum [74,75]. Inactivation of *Wnt4* results in the absence of kidneys [76], masculinization of mutant females (absence of the Müllerian duct and continued development of the Wolffian duct) [77], and defects in mammary gland morphogenesis during pregnancy [78]. Targeted knockout of *Wnt7a* also has pleiotropic effects, including ventralization of the limbs

Table 2

Developmental functions of mouse Wnt genes

Gene	Natural allele	Phenotype of knockout or other functions	References
<i>Wnt1</i>	<i>swaybig</i>	Loss of a portion of the midbrain and cerebellum Deficiency in dorsal neural-tube derivatives, including neural-crest cells in double knockout with <i>Wnt3a</i>	[74,75,129,130] [131]
<i>Wnt2</i>		Placental defects	[132]
<i>Wnt3</i>		Defects in axis formation and gastrulation Defects in hair growth and structure	[84] [133,134]
<i>Wnt3a</i>	<i>vestigial tail</i>	Defects in somite and tailbud development Deficiency in dorsal neural-tube derivatives, including neural crest cells in double knockout with <i>Wnt1</i> Loss of hippocampus	[102,135-137] [131] [138]
<i>Wnt4</i>		Defects in kidney development Defects in female development; absence of Müllerian duct, ectopic synthesis of testosterone in females Defects in mammary gland morphogenesis	[76] [77] [78]
<i>Wnt5a</i>		Truncated limbs, shortened anterior-posterior axis, reduced number of proliferating cells	[139]
<i>Wnt7a</i>	<i>postaxial hemimegal</i>	Defects in limb polarity Female infertility due to failure of Müllerian duct regression Defects in uterine patterning Defects in synapse maturation in the cerebellum	[79] [80,140] [141] [81]
<i>Wnt7b</i>		Placental defects	[142]
<i>Wnt10b</i>		Inhibition of adipogenesis	[143]

[79], female infertility due to failure of Müllerian-duct regression [80], and a delay in the morphological maturation of glomerular rosaries in the cerebellum [81].

Overexpression and antisense 'knockdown' analyses in *Xenopus* have shown that the Wnt/ β -catenin pathway is required for the specification of dorsal cell fates [82]. A debate is ongoing, however, over whether a maternal Wnt ligand is required to activate this pathway in dorsal cells. In support of a role for a Wnt ligand, a recent study has shown that *Xfzd7* is important for establishing dorsal cell fates [83], thereby implicating a Wnt ligand in this process. Furthermore, targeted knockout of *Wnt3* in mice results in defects in axis formation and gastrulation, suggesting a conserved role for Wnts in regulating the establishment of the dorsal-ventral axis in vertebrates [84]. On the other hand, overexpression of a dominant-negative form of *Xunt8* in oocytes does not suppress formation of dorsal cell fates, arguing against the requirement for a maternal Wnt in axis specification [10]. Further studies are necessary to resolve the role of Wnts in vertebrate early axial development.

In flies, Wnt signaling has a variety of functions during development. The *ug* gene is required for cell-fate choices in the ventral epidermis during embryogenesis, as well as for many other functions, and *DWnt2* is required for testis and adult muscle development [17]. In *C. elegans*, genetic analyses have defined a number of roles for Wnts, including establishment of polarity and endodermal cell fates in the early embryo and regulation of cell migration, among many others [85]. A comprehensive list of Wnt genes and their mutant phenotypes in vertebrates and invertebrates can be found at the Wnt gene homepage [5].

Wnt signaling and cancer

In addition to the many roles for Wnt signaling during development and in adult tissues, it is also involved in tumorigenesis in humans [59,64]. Although mutation or misexpression of a Wnt gene has not been linked directly to cancer in humans, mutation of several intracellular components of the Wnt/ β -catenin pathway is thought to be critical in many forms of cancer. Most notably, patients with familial adenomatous polyposis (FAP) develop multiple intestinal adenomas early in life and have germline mutations in the APC gene. In addition, mutation of APC is associated with more than 80% of sporadic colorectal adenomas and carcinomas. More than 95% of germline and somatic mutations of the APC gene are nonsense mutations that result in the synthesis of a truncated protein lacking the region of APC that is important for its function in the destruction complex. Significantly, these truncations in APC remove binding sites for β -catenin and Axin, as well as putative phosphorylation sites for GSK3; as a result, the mutant APC protein cannot efficiently promote degradation of β -catenin. Mutations in the third exon of the human β -catenin gene (*CTNNB1*) that make it refractory to phosphorylation-dependent degradation and

lead to inappropriate accumulation of β -catenin have also been identified in a large number of primary human cancers (see [64] for a table of β -catenin mutations in human cancers). Interestingly, mutations in *CTNNB1* and APC are rarely found in the same tumor; for example, in colon cancer, in which the vast majority of tumors have mutations in APC, the overall frequency of *CTNNB1* mutations is relatively low, but colorectal tumors lacking APC mutations are much more likely to have mutations in *CTNNB1*. Recently, Axin has also been shown to act as a tumor suppressor; mutations in the *Axin1* gene have been found in human hepatocellular cancers [86]. Importantly, mutations in *Axin1* and *CTNNB1* found in hepatocellular carcinomas also show mutual exclusivity similar to that seen for APC and *CTNNB1* in colon cancers. Together, these data strongly argue that mutations resulting in the stabilization of β -catenin can promote cancer in many tissue types.

Frontiers

The large number of Wnt genes and the many roles that Wnt signaling plays in development and human disease pose many unresolved issues for researchers. One of the major unanswered questions is the specificity of interactions between different Wnt ligands and Fzd receptors and also which downstream pathways these many different ligand-receptor pairs stimulate. It also remains unclear how Wnt signals are transduced by the Fzd-LRP receptor complex and what role proteoglycans play in this process. Inside the cell, many questions regarding the transduction of Wnt signals remain, including how receptor activation stimulates Dsh and how Dsh discriminates between different Wnt signals to activate either the Wnt/ β -catenin or the Wnt/polarity pathway. Furthermore, many roles of Wnts during development remain to be determined. This challenge will require detailed analyses of knockout mice, in addition to biochemical, cell-biological and genetic analyses in other model systems, to characterize the functions of Wnts and the signaling pathways they use during embryogenesis. Finally, the identification and characterization of mutations in Wnt-pathway genes involved in human disease is ongoing and these studies, together with a greater knowledge of the molecular mechanism of Wnt signal transduction, promise future clinical therapies for devastating human afflictions such as colon cancer. Thus, although there is so much still to learn, the importance and widespread occurrence of Wnt signaling guarantees the rapid increase in our understanding of the normal and abnormal functions of the Wnts.

References

1. The Genome Database (<http://gdbwww.gdb.org/gdb/>)
The Genome Database (GDB) is the official central repository for genomic mapping data resulting from the Human Genome Initiative.
2. SOURCE
(<http://genom-www4.stanford.edu/cgi-bin/SMD/source/sourceSearch>)
The Stanford Online Universal Resource for Clones and ESTs (SOURCE) compiles information from several publicly accessible databases, including

<http://genomebiology.com/2001/3/1/reviews/3001.9>

- UniGene, dbEST, SWISSPROT, GeneMap99, RIKEN, GeneCards and LocusLink to provide a scientific resource that pools publicly available data commonly sought after for any gene. GenBank accession number, or gene.
3. GenBank [<http://www.ncbi.nlm.nih.gov/Genbank/index.html>]
Database of DNA and protein sequences.
 4. GeneCards [<http://genome-www.stanford.edu/genecards/index.html>]
GeneCards™ is a database of human genes, their products and their involvement in diseases.
 5. The Wnt gene homepage
[<http://www.stanford.edu/~nusse/wntwindow.html>]
An excellent resource for information on genes involved in Wnt signal transduction. The site provides comprehensive information on Wnt ligands and Fzd receptors as well as genes involved in Wnt/ β -catenin signaling.
 6. LocusLink [<http://www.ncbi.nlm.nih.gov/LocusLink>]
LocusLink provides curated information for human, fruit fly, mouse, rat and zebrafish.
 7. Nusse R: An ancient cluster of Wnt paralogues *Trends Genet* 2001, 17:443.
This paper discusses the conserved arrangement of a group of Wnt genes in the human and *Drosophila* genomes.
 8. Entrez Genome View [http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/hum_srch?chr=hum_dir.inf&query]
The NCBI Map Viewer provides graphical displays of features on NCBI's assembly of human genomic sequence data as well as cytogenetic, genetic, physical, and radiation hybrid maps.
 9. Du SJ, Purcell SM, Christian JL, McGraw LL, Moon RT: Identification of distinct classes and functional domains of Wnts through expression of wild-type and chimeric proteins in *Xenopus* embryos. *Mol Cell Biol* 1995, 15:2625-2634.
The authors investigated whether distinct domains of XWnt8 and XWnt5a were required to elicit distinct functions. They found that the carboxy-terminal of these Wnts were sufficient to produce specific phenotypes and marker gene expression.
 10. Hoppler S, Brown JD, Moon RT: Expression of a dominant-negative Wnt blocks induction of MyoD in *Xenopus* embryos. *Genes Dev* 1996, 10:2805-2817.
This paper shows that expression of a carboxy-terminal truncation mutant of XWnt8 acts as a dominant-negative in early *Xenopus* embryos in a cell non-autonomous manner, suggesting that it might act by preventing the association of wild-type XWnt8 with its receptor.
 11. Dierick H, Bejsovec A: Cellular mechanisms of wingless/Wnt signal transduction. *Curr Top Dev Biol* 1999, 43:153-190.
A comprehensive review of Wnt signal transduction, focusing on the signaling mechanism of *Drosophila* Wg.
 12. van den Heuvel M, Harryman-Samos C, Klingensmith J, Perrimon N, Nusse R: Mutations in the segment polarity genes *wingless* and *porcupine* impair secretion of the Wingless protein. *EMBO J* 1993, 12:5293-5302.
Several embryonic-lethal alleles of *wg* produce mutant Wg protein that is retained inside producing cells. A similar abnormal distribution of wild-type Wg protein was also seen in embryos mutant for the segment polarity gene *porcupine* suggesting that Porcupine plays a role in regulating Wg biosynthesis or secretion.
 13. Cadigan KM, Nusse R: wingless signaling in the *Drosophila* eye and embryonic epidermis. *Development* 1996, 122:2801-2812.
Shows that Wg acts in a paracrine manner and that porcupine is required in mediating Wg signaling.
 14. Kadowaki T, Wilder E, Klingensmith J, Zachary K, Perrimon N: The segment polarity gene *porcupine* encodes a putative multi-transmembrane protein involved in wingless processing. *Genes Dev* 1996, 10:3116-3128.
The authors present the cloning and sequence of the *porcupine* gene and show that the Porcupine protein localizes to the endoplasmic reticulum and plays a role in the biosynthetic processing of Wg.
 15. Rocheleau CE, Downs WD, Lin R, Witmann C, Bei Y, Cha YH, Ali M, Priess JR, Mello CC: Wnt signaling and an APC-related gene specify endoderm in early *C. elegans* embryos. *Cell* 1997, 90:707-716.
See [16].
 16. Thorpe CJ, Schlesinger A, Carter JC, Bowerman B: Wnt signaling polarizes an early *C. elegans* blastomere to distinguish endoderm from mesoderm. *Cell* 1997, 90:695-705.
Data in this paper and [15] demonstrate a requirement for Wnt signaling in the determination of the endodermal lineage in early *C. elegans* embryos. In addition to the role of Wnt in cell fate determination, Thorpe et al. also describe a role for Wnt in regulating mitotic spindle orientation in the early embryo.
 17. Cadigan KM, Nusse R: Wnt signaling: a common theme in animal development. *Genes Dev* 1997, 11:3286-3305.
A comprehensive review discussing the role of Wnt signaling during development, focusing on the function of Wnt genes in development and the molecular mechanism of Wnt signal transduction.
 18. Mason JO, Kitajewski J, Varmus HE: Mutational analysis of mouse Wnt-1 identifies two temperature-sensitive alleles and attributes of Wnt-1 protein essential for transformation of a mammary cell line. *Mol Biol Cell* 1992, 3:521-533.
Shows that mutation of all four potential glycosylation sites in Wnt1 does not abolish its ability to promote transformation of C3/15G mouse mammary epithelial cells.
 19. Bradley AS, Brown AM: The proto-oncogene *int-1* encodes a secreted protein associated with the extracellular matrix. *EMBO J* 1990, 9:1569-1575.
The authors expressed *int-1* (Wnt1) in fibroblasts and found that it could not be detected in the culture medium but instead was found in tight association with the extracellular matrix. They also presented evidence that Wnt1 can bind heparin *in vitro*, suggesting that Wnt proteins may associate with glycosaminoglycans.
 20. Relehsman F, Smith L, Cumberland S: Glycosaminoglycans can modulate extracellular localization of the wingless protein and promote signal transduction. *J Cell Biol* 1996, 135:819-827.
This paper presents evidence that Wg protein associates with glycosaminoglycans (heparan sulfate and chondroitin sulfate) on the surface of producing and receiving cells. In addition, perturbation of glycosaminoglycan synthesis was found to greatly reduce Wg signaling, suggesting that glycosaminoglycans are required for optimal Wnt signaling.
 21. Shibamoto S, Higano K, Takada R, Ito F, Takeichi M, Takada S: Cytoskeletal reorganization by soluble Wnt3a protein signaling. *Genes Cells* 1998, 3:659-670.
Takada and colleagues describe the production and activity of Wnt3a-conditioned media, a source of soluble Wnt protein that can be used in different experimental analyses.
 22. van Leeuwen F, Samos CH, Nusse R: Biological activity of soluble wingless protein in cultured *Drosophila* imaginal disc cells. *Nature* 1994, 368:342-344.
This paper describes the production of Wg-conditioned media, showing that Wg, and by analogy other Wnt proteins, can act as soluble extracellular signaling molecules.
 23. van den Heuvel M, Nusse R, Johnston P, Lawrence PA: Distribution of the wingless gene product in *Drosophila* embryos: a protein involved in cell-cell communication. *Cell* 1989, 59:739-749.
The authors demonstrate that Wg protein can be found in producing cells, in intercellular regions in association with the plasma membrane, and in multivesicular bodies inside Wg responding cells. The latter observation suggests that endocytosis of Wg may play a role in Wnt signaling (see [24]).
 24. Gonzalez F, Swales L, Bejsovec A, Sklar H, Martinez Ariza A: Secretion and movement of wingless protein in the epidermis of the *Drosophila* embryo. *Mech Dev* 1991, 35:43-54.
The Wg protein can be found several cell diameters from its source, indicating that it is a secreted protein that can act at a distance from producing cells. In addition, it was found that Wg can be endocytosed by receiving cells.
 25. Bejsovec A, Wieschaus E: Signaling activities of the *Drosophila* wingless gene are separately mutable and appear to be transduced at the cell surface. *Genetics* 1995, 139:309-320.
Data in this paper and [26] demonstrate that inhibition of endocytosis compromises Wg signaling and perturbs Wg distribution. Together these data indicate that internalization of Wnt ligands may play a critical role in controlling activation of downstream signaling and governing the range of action of Wnt ligands.
 26. Moline MM, Southern C, Bejsovec A: Directionality of wingless protein transport influences epidermal patterning in the *Drosophila* embryo. *Development* 1999, 126:4375-4384.
See [25].
 27. Dubois L, Lecourtis M, Alexandre C, Hirt E, Vincent JP: Regulated endocytic routing modulates wingless signalling in *Drosophila* embryos. *Cell* 2001, 105:613-624.
Regulated lysosomal degradation of Wg protein is one mechanism that functions to control the distribution of active Wg ligand, and activation of the epidermal growth factor receptor facilitates degradation of Wg.

28. Greco V, Hannus M, Eaton S: **Argosomes: a potential vehicle for the spread of morphogens through epithelia.** *Cell* 2001, 106:633-645.
This paper describes a novel endocytic compartment in *Drosophila* embryos termed argosomes that may represent a specialized exovesicle important for transcytosis and movement of signaling molecules through epithelia. Wg protein was found to co-localize with argosomes, suggesting that argosomes may represent a novel vehicle for the transport of Wnt ligands through epithelia.
29. Simmonds AJ, dosSantos G, Livne-Bar I, Krause HM: **Apical localization of wingless transcripts is required for wingless signaling.** *Cell* 2001, 105:197-207.
Using high-resolution *in situ* hybridization analyses, the authors show that wingless transcripts are apically localized in several tissues during *Drosophila* development. This polarized distribution was dependent on two cis-acting elements found in the 3' UTR of the wingless transcript. Mutation of these elements resulted in mis-localization Wg protein as well as a reduction in Wg signaling activity.
30. Wilkie GS, Davis I: ***Drosophila* wingless and pair-rule transcripts localize apically by dynein-mediated transport of RNA particles.** *Cell* 2001, 105:209-219.
This paper demonstrates that wingless transcripts assemble into cytoplasmic particles that are transported to apical regions of the cell via microtubules and dynein motors.
31. Beljovet A: **Wnt signaling: an embarrassment of receptors.** *Curr Biol* 2000, 10:R919-R922.
This review and [32] discuss the recent identification of LRP as a Wnt co-receptor.
32. Pandur P, Kuhl M: **An arrow for wingless to take-off.** *BioEssays* 2001, 23:207-210.
See [31].
33. Dann CE, Hsieh JC, Ratzier A, Sharma D, Nathans J, Leahy DJ: **Insights into Wnt binding and signaling from the structures of two Frizzled cysteine-rich domains.** *Nature* 2001, 412:86-90.
The structure of the cysteine-rich domains (CRDs) of mouse Fzd8 and secreted Fzd-related protein 3 (sFRP3). The CRD was shown to form a novel protein fold, and the design and interpretation of CRD mutations identified a Wnt-binding site. The CRDs were also found to exhibit a conserved dimer interface that may be a feature of Wnt signaling.
34. Sheldahl LC, Park M, Malbon CC, Moon RT: **Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner.** *Curr Biol* 1999, 9:695-698.
This paper builds on a previous observation that signaling by Wnt5a and Fzd2 leads to an increase in intracellular Ca^{2+} and demonstrates that Wnt5a and Fzd2 also activate PKC in *Xenopus* embryos. The authors also show that distinct subsets Wnt ligands and Fzd receptors stimulate either the Wnt/ Ca^{2+} or the Wnt/ β -catenin pathway in *Xenopus*.
35. Liu X, Liu T, Slusarski DC, Yang-Snyder J, Malbon CC, Moon RT, Wang H: **Activation of a frizzled-2/ β -adrenergic receptor chimera promotes Wnt signaling and differentiation of mouse F9 teratocarcinoma cells via G_{12} and G_{13} .** *Proc Natl Acad Sci USA* 1999, 96:14383-14388.
The authors engineered a rat Fzd2 chimera responsive to β -adrenergic agonist by fusing the ligand-binding domains of the $\beta(2)$ -adrenergic receptor to the intracellular loops of Fzd2. Isoproterenol-induced activation of the Fzd2 chimera in F9 embryonic teratocarcinoma cells was blocked by pertussis toxin and by oligodeoxynucleotide antisense to G_{12} , G_{13} and G_{14} demonstrating the involvement of two pertussis toxin-sensitive G proteins for signaling by the Fzd2 receptor.
36. Liu T, Liu X, Wang H, Moon RT, Malbon CC: **Activation of rat frizzled-1 promotes Wnt signaling and differentiation of mouse F9 teratocarcinoma cells via pathways that require G α_{12} (q) and G α_{13} (o) function.** *J Biol Chem* 1999, 274:33539-33544.
This paper demonstrated that stimulation of F9 teratocarcinoma cells expressing rat Fzd1 with *Xenopus* Wnt8-conditioned media results in differentiation of the cells into primitive endoderm. Fzd1/Wnt8-dependent differentiation could be blocked by pertussis toxin, depletion of G_{12} or G_{13} , inhibition of PKC, and inhibition of mitogen-activated protein kinase (MAPK), suggesting that signaling by Fzd1 in F9 cells involves activation of heterotrimeric G-proteins, PKC and MAPK.
37. Kuhl M, Sheldahl LC, Malbon CC, Moon RT: **Ca^{2+} /calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in *Xenopus*.** *J Biol Chem* 2000, 275:12701-12711.
This paper demonstrates that expression of a subset of Wnt ligands (Wnt5a and Wnt11) and Fzd receptors (including rat Fzd2) in *Xenopus* embryos leads to the stimulation of CamKII. Using chimeric β -adrenergic/Fzd2 receptors, the authors also show that activation of CamKII occurs within 10 minutes following receptor stimulation and is sensitive to pertussis toxin.
38. Liu T, DeCostanzo AJ, Liu X, Wang H, Hallagan S, Moon RT, Malbon CC: **G protein signaling from activated rat frizzled-1 to the β -catenin-Lef-Tcf pathway.** *Science* 2001, 292:1718-1722.
This paper shows that stimulation of a chimeric β -adrenergic/Fzd1 receptor expressed in mouse F9 teratocarcinoma cells with isoproterenol results in the stabilization of β -catenin and activation of a β -catenin-responsive reporter gene. Both of these effects could be blocked by pertussis toxin, indicating that heterotrimeric G proteins may be involved in transducing signals from Fzd1 to the Wnt/ β -catenin pathway.
39. Tamai K, Semenov M, Kato Y, Spokony R, Liu C, Katsuyama Y, Hess F, Saint-Jeannet JP, He X: **LDL-receptor-related proteins in Wnt signal transduction.** *Nature* 2000, 407:530-535.
The authors demonstrate that LRP6 can act as a Wnt receptor in *Xenopus* embryos. Overexpression of LRP6 in *Xenopus* resulted in axis duplication and activation of Wnt-responsive genes while overexpression of a truncated form of LRP6 blocked Wnt activity in the same assays. Furthermore, LRP6 can bind Wnt and interacts with Fzd in a Wnt-dependent manner.
40. Pinson KL, Brennan J, Monkley S, Avery BJ, Skarnes WC: **An LDL-receptor-related protein mediates Wnt signalling in mice.** *Nature* 2000, 407:535-538.
This paper provides evidence that LRP6 can act as a Wnt receptor in mice. Embryos homozygous for a mutation in the *LRP6* gene exhibit developmental defects that are a striking composite of those caused by mutations in individual *Wnt* genes. Furthermore, the authors show a genetic enhancement of the vestigial tail (*Wnt3d*) phenotype in mice lacking one functional copy of *LRP6*.
41. Wehrli M, Dougan ST, Caldwell K, O'Keefe L, Schwartz S, Yaizel-Ohayon D, Schejter E, Tomlinson A, DiNardo S: **arrow encodes an LDL-receptor-related protein essential for Wingless signalling.** *Nature* 2000, 407:527-530.
This paper demonstrates that the *arrow* gene is necessary for all Wg signaling events in *Drosophila*. The authors also provide genetic evidence that *arrow* gene function is essential in cells receiving Wg input and that it acts upstream of Dsh.
42. Mao J, Wang J, Liu B, Pan W, Farr GH 3rd, Flynn C, Yuan H, Takada S, Krimelman D, U L, et al: **Low-density lipoprotein receptor-related protein-5 binds to Axin and regulates the canonical Wnt signaling pathway.** *Mol Cell* 2001, 7:801-809.
This paper shows that the intracellular domain of LRP5 binds Axin. Wnt signals were found to cause recruitment of Axin to the membrane and enhanced the interaction of Axin with LRP5. Together, these data suggest that activation of the Wnt/ β -catenin pathway may involve direct interaction of Axin with the Wnt receptor complex.
43. Lin X, Perrimon N: **Dally cooperates with *Drosophila* Frizzled 2 to transduce Wingless signalling.** *Nature* 1999, 400:281-284.
The authors show that mutation of *dally*, a member of the glypican family of heparan sulfate proteoglycans, results in phenotypes similar to partial loss of wingless function. Loss of *dally* was also found to enhance loss-of-function *DFzd2* phenotype.
44. Tsuda M, Kamimura K, Nakato H, Archer M, Sotaz W, Fox B, Humphrey M, Olson S, Futch T, Kaluz V, et al: **The cell-surface proteoglycan Dally regulates Wingless signalling in *Drosophila*.** *Nature* 1999, 400:276-280.
Similar to the results reported in [43] this paper describes genetic evidence that *dally* plays a role in the reception of Wg signals.
45. Binari RC, Staveley BE, Johnson WA, Godavari R, Sasisekharan R, Manoukian AS: **Genetic evidence that heparin-like glycosaminoglycans are involved in wingless signalling.** *Development* 1997, 124:2623-2632.
This paper shows that injection of heparinase into *Drosophila* embryos results in the degradation of heparin-like glycosaminoglycans and a wingless-like cuticular phenotype, suggesting the proteoglycans are involved in Wnt signaling.
46. Hacker U, Lin X, Perrimon N: **The *Drosophila* sugarless gene modulates Wingless signaling and encodes an enzyme involved in polysaccharide biosynthesis.** *Development* 1997, 124:3565-3573.
This paper describes genetic evidence that the *sugarless* gene, a *Drosophila* homolog of vertebrate UDP-glucose dehydrogenase, is required for optimal Wg signaling. UDP-glucose dehydrogenase is essential for the biosynthesis of various proteoglycans, suggesting that

<http://genomebiology.com/2001/3/1/reviews/3001.1>

- proteoglycans play an important role in the production or reception of Wnt signals.
47. Haerry TE, Heslop TR, Marsh JL, O'Connor MB: Defects in glucuronate biosynthesis disrupt Wingless signaling in *Drosophila*. *Development* 1997, 124:3055-3064.
The authors describe the identification and characterization of the *Drosophila* *suppenkasper* (*sko*) gene that encodes a UDP-glucose dehydrogenase required for production of glucuronic acid. Genetic analyses show that the phenotype *sko* mutant embryos resemble that of wingless deficient embryos and that *sko* interacts with both wingless and dishevelled.
 48. Dhoot GK, Gustafsson MK, Ai X, Sun W, Standford DM, Emerson CP Jr.: Regulation of Wnt signaling and embryo patterning by an extracellular sulfatase. *Science* 2001, 293:1663-1666.
The identification of *QSOX1*, an avian ortholog of an evolutionarily conserved protein family related to heparin-specific N-acetyl glucosaminyl sulfatases. In cultured C2C12 myogenic progenitor cells *QSOX1* was found to facilitate Wnt signaling, suggesting that *QSOX1* can modulate Wnt signals by desulfation of cell-surface proteoglycans.
 49. Moon RT, Brown JD, Yang-Snyder JA, Miller JR: Structurally related receptors and antagonists compete for secreted Wnt ligands. *Cell* 1997, 88:725-728.
This review summarizes the discovery and function of FRPs with a focus on the role of FRPs during early *Xenopus* development.
 50. Hsieh JC, Kodjabachian L, Rebbert ML, Ratzner A, Smallwood PM, Samos CH, Nusse R, Dawid IB, Nathans J: A new secreted protein that binds to Wnt proteins and inhibits their activities. *Nature* 1999, 398:431-436.
The authors describe the identification of Wnt-inhibitory factor 1 (WIF-1), a secreted Wnt antagonist, and show that overexpression of WIF-1 in *Xenopus* embryos perturbs somitogenesis.
 51. Piccolo S, Agius E, Lyons L, Bhattacharyya S, Grunz H, Bouwmeester T, De Robertis EM: The head inducer Cerberus is a multifunctional antagonist of Nodal, BMP and Wnt signals. *Nature* 1999, 397:707-710.
This paper shows that the Cerberus protein can bind to Nodal, BMP and Wnt proteins via independent sites, suggesting that it functions as a multivalent growth-factor antagonist. Based on overexpression experiments in *Xenopus*, the authors propose that Cerberus functions to block Nodal, BMP, and Wnt signals involved in trunk formation thereby promoting head formation in anterior regions of the embryo.
 52. Nusse R: Developmental biology. Making head or tail of Dickkopf. *Nature* 2001, 411:255-256.
This comment article summarizes data presented in [53].
 53. Ballico A, Liu G, Yaniv A, Gazit A, Aaronson SA: Novel mechanism of Wnt signaling inhibition mediated by Dickkopf-1 interaction with LRP6/Arrow. *Nat Cell Biol* 2001, 3:683-686.
This paper demonstrates that Dickkopf-1 (Dkk1), a secreted Wnt antagonist, blocks Wnt signaling by binding to the extracellular domain of the Wnt receptors LRP6 and LRP6.
 54. Semenov MV, Tamai K, Brodt BK, Kuhl M, Sokol S, He X: Head Inducer Dickkopf-1 is a ligand for Wnt coreceptor LRP6. *Curr Biol* 2001, 11:951-961.
The authors show that Dickkopf-1 (Dkk-1) is a high-affinity LRP6 ligand that inhibits Wnt signaling by blocking Wnt-induced Fzd-LRP6 complex formation.
 55. Wu W, Glinks A, Delus H, Niehrs C: Mutual antagonism between dickkopf1 and dickkopf2 regulates Wnt/beta-catenin signalling. *Curr Biol* 2000, 10:1611-1614.
This paper shows that dickkopf2 (Dkk2) and Wnt act synergistically in *Xenopus* embryos to activate the Wnt/beta-catenin pathway. Thus, unlike other members of the DKK family that act as Wnt antagonists Dkk2 appears to function as a stimulatory co-factor for Wnt signaling.
 56. Adler PN, Lee H: Fizzled signaling and cell-cell interactions in planar polarity. *Curr Opin Cell Biol* 2001, 13:635-640.
This review provides a current summary of the role of Fzd and Dsh in regulating planar polarity during *Drosophila* development.
 57. Boutsos M, Modzik M, Dishevelled: at the crossroads of divergent intracellular signaling pathways. *Mech Dev* 1999, 83:27-37.
This review provides a summary of the function of Dsh in Wnt signaling and discusses how Dsh discriminates between different Wnt inputs to modulate distinct downstream cellular responses.
 58. Kuhl M, Sheldahl LC, Park M, Miller JR, Moon RT: The Wnt/Ca2+ pathway: a new vertebrate Wnt signaling pathway takes shape. *Trends Genet* 2000, 16:279-283.
This review provides a synopsis of our current understanding of the Wnt/Ca2+ pathway and presents an interesting table describing the apparent mutually exclusive ability of different Fzd receptors to stimulate either the Wnt/beta-catenin or Wnt/Ca2+ pathways.
 59. Miller JR, Hocking AM, Brown JD, Moon RT: Mechanism and function of signal transduction by the Wnt/beta-catenin and Wnt/Ca2+ pathways. *Oncogene* 1999, 18:7860-7872.
This review provides a detailed summary of our current understanding of the molecular mechanisms underlying signaling through the Wnt/beta-catenin or Wnt/Ca2+ pathways. Particular attention is paid to the function and regulation of the Axin/APC/GSK3 destruction complex and the involvement of Wnt pathway genes in human cancer.
 60. Science's STKE Connections Map [<http://stke.sciencemag.org/cm/>]
This website is an excellent source of up-to-date information on a variety of cell signaling topics and includes an interactive connections map for several Wnt pathways.
 61. A Pond in Seattle: *Xenopus* and Zebrafish Research in the lab of Dr. Randall Moon [<http://faculty.washington.edu/rmoon/>]
This website contains several Flash animated movies of Wnt signaling and is an excellent source for DNA constructs useful for studying Wnt signaling.
 62. Wnt World [<http://www.gcd.med.uminn.edu/millerlab/Wnt/Wntworld.html>]
This website, maintained by my lab, is a new venture aimed at providing up-to-date information on the mechanism of Wnt signaling and the function of Wnt signaling during development and in human disease.
 63. Wodarz A, Nusse R: Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol* 1998, 14:59-88.
This review provides a comprehensive view of the role of Wnt signaling during development.
 64. Polakis F: Wnt signalling and cancer. *Genes Dev* 2000, 14:1837-1851.
This review provides an excellent synopsis of our current understanding of the role of Wnt signaling in human cancer. It also contains a table describing beta-catenin mutations found in various human cancers.
 65. Torres MA, Yang-Snyder JA, Purcell SM, Doherty AA, McGraw LL, Moon RT: Activities of the Wnt-1 class of secreted signaling factors are antagonized by the Wnt-5A class and by a dominant negative cadherin in early *Xenopus* development. *J Cell Biol* 1996, 133:1123-1137.
The authors demonstrate that overexpression of XWnt5a can inhibit signaling by Xwnt1 in *Xenopus* embryos through a mechanism that may involve changes in cell-cell adhesion.
 66. Heisenberg CP, Tada M, Rauch GJ, Saude L, Concha ML, Gelsler R, Seemle DL, Smith JC, Wilson SV: Silberblick/Wnt11 mediates convergent extension movements during zebrafish gastrulation. *Nature* 2000, 405:76-81.
This paper describes the characterization of the *silberblick*/*Wnt11* gene in zebrafish. The authors demonstrate that *silberblick*/*Wnt11* is required for convergent-extension movements and that overexpression of a truncated form of Dsh active in Wnt/polarity signaling but not Wnt/beta-catenin signaling can compensate for the loss of *silberblick*/*Wnt11* function.
 67. Tada M, Smith JC: Xwnt11 is a target of *Xenopus* Brachyury regulation of gastrulation movements via Dishevelled, but not through the canonical Wnt pathway. *Development* 2000, 127:2227-2238.
The authors demonstrate that overexpression of a dominant-negative form of XWnt11 in *Xenopus* embryos inhibits convergent extension movements. Co-expression of wild-type Dsh or a truncated form of Dsh that cannot signal through the Wnt/beta-catenin pathway can overcome this inhibitory effect.
 68. Wallingford JB, Rowling BA, Vogeli KM, Rothbacher U, Fraser SE, Harland RM: Dishevelled controls cell polarity during *Xenopus* gastrulation. *Nature* 2000, 405:81-85.
This paper demonstrates that overexpression of a truncated form of Dsh that inhibits Wnt/polarity signaling, but not Wnt/beta-catenin signaling, disrupts convergent extension movements in *Xenopus*. The authors provide a detailed analysis of the effects of the truncated form of Dsh on cell movements and demonstrate that Dsh regulates the polarization of cells along the medial-lateral axis as well as the dynamics and polarity of cellular protrusions during gastrulation.
 69. Marsden M, DeSimone DW: Regulation of cell polarity, radial intercalation and epiboly in *Xenopus*: novel roles for integrin and fibronectin. *Development* 2001, 128:3635-3647.
This paper shows that integrin-dependent binding of blastocoel roof cells to fibronectin is sufficient to drive membrane localization of Dsh-GFP, suggesting that a convergence of integrin and Wnt signaling pathways acts to regulate morphogenesis in *Xenopus* embryo.

70. Wallingford JB, Harland RM: **Xenopus Dishevelled signaling regulates both neural and mesodermal convergent extension: parallel forces elongating the body axis.** *Development* 2001, 128:2581-2592.
This paper shows that spatially restricted expression of Dsh mutants that block Wnt/Polarity signaling, but not Wnt/ β -catenin signaling, to neural or mesodermal tissues inhibited either neural or mesodermal convergent extension. Targeted expression of other Wnt signaling antagonists also inhibited neural convergent extension in whole embryos without affecting cell fate, suggesting that Wnt/Polarity signaling regulates morphogenesis of both mesodermal and neural tissues during vertebrate development.
71. Adler PN, Taylor J: **Asymmetric cell division: plane but not simple.** *Curr Biol* 2001, 11:R233-R236.
This review discusses the role of Fzd receptors in the regulation of asymmetric cell divisions in *Drosophila* embryos.
72. Surut D: **Planar polarity: getting ready to ROCK.** *Curr Biol* 2001, 11:R506-R509.
This review summarizes recent advances in our understanding of how Fzd and Dsh regulate planar polarity in *Drosophila* focusing on the role of the rho-associated kinase ROCK in this process.
73. He X, Saint-Jeanet JP, Wang Y, Nathans J, Dawid I, Varmus H: **A member of the Frizzled protein family mediates axis induction by Wnt3A.** *Science* 1997, 273:1652-1654.
The authors describe experiments demonstrating that overexpression of Wnt3a in combination with human FZDS promotes signaling through the Wnt/ β -catenin pathway suggesting that the specificity of cellular responses to different Wnt signals is regulated by the repertoire of Fzd receptors present on the cell surface of responding cells.
74. McMahon AP, Bradley A: **The Wnt-1 (Int-1) proto-oncogene is required for development of a large region of the mouse brain.** *Cell* 1990, 62:1073-1085.
This paper describes the knockout of Wnt1 in the mouse and demonstrates that Wnt1 is required for the development of the neocortex and cerebellum.
75. Thomas KR, Musci TS, Neumann PE, Capocchi MR: **Swaying is a mutant allele of the proto-oncogene Wnt-1.** *Cell* 1991, 67:969-976.
The authors show that swaying phenotype is caused by deletion of a single base pair from Wnt1 that results in premature termination of translation, eliminating the carboxy-terminal half of the Wnt1 protein.
76. Stark K, Valino S, Vassileva G, McMahon AP: **Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4.** *Nature* 1994, 372:679-683.
This paper describes the expression pattern of Wnt4 and the knockout phenotype of embryos lacking Wnt4 activity. Wnt4 mutant mice fail to form pretubular cell aggregates in the developing kidney, suggesting that Wnt4 regulates the mesenchyme to epithelial transition that underlies nephron development.
77. Valino S, Helkkila M, Kispert A, Chin N, McMahon AP: **Female development in mammals is regulated by Wnt-4 signalling.** *Nature* 1999, 397:405-409.
This paper demonstrates that Wnt4 is required for the development of sexual dimorphism. Females lacking Wnt4 fail to form the Müllerian duct while the Wolffian duct continues to develop.
78. Brisken C, Helman A, Chavarría T, Elenbaas B, Tan J, Dey SK, McMahon JA, McMahon AP, Weinberg RA: **Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling.** *Genes Dev* 2000, 14:650-654.
The authors perform transplantation studies to demonstrate that mammary tissue lacking Wnt4 fails to undergo side branching during pregnancy. They also found that Wnt4 expression is regulated by progesterone. Together these data suggest that Wnt signaling is necessary to mediate progesterone function during mammary gland morphogenesis.
79. Parr BA, McMahon AP: **Dorsalizing signal Wnt-7a required for normal polarity of D-V and A-P axes of mouse limb.** *Nature* 1993, 374:350-353.
This paper describes the knockout phenotype of mice lacking Wnt7a focusing on the role of Wnt7a in the limb. Mutant embryos display defects in limb patterning characterized by a dorsal-to ventral transformation of cell fate, indicating that Wnt7a is a dorsalizing signal.
80. Parr BA, McMahon AP: **Sexually dimorphic development of the mammalian reproductive tract requires Wnt-7a.** *Nature* 1998, 395:707-710.
This paper demonstrates that Wnt7a is required for establishment of sexual dimorphism. Male mice lacking Wnt7a fail to undergo regression of the Müllerian duct due to the absence of the receptor for Müllerian-inhibiting substance. The authors also show that mutation of Wnt7a affects development of female-specific tissues. Wnt7a-deficient females are infertile because of abnormal development of the oviduct and uterus.
81. Hall AC, Lucas FR, Salinas PC: **Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling.** *Cell* 2000, 100:525-535.
This paper shows that the WNT antagonist sFRP-1 can block endogenous signals produced by granule cells that induce axon and growth cone remodeling in mossy fibers. In contrast, WNT7a, which is expressed by granule cells, can mimic the endogenous signal.
82. Moon RT, Kimelman D: **From cortical rotation to organizer gene expression: toward a molecular explanation of axis specification in Xenopus.** *BioEssays* 1998, 20:536-545.
A comprehensive review summarizing the role of Wnt signaling in the establishment of the dorsal-ventral axis in *Xenopus*.
83. Sumanas S, Srege P, Heasman J, Ekker SC: **The putative wnt receptor Xenopus frizzled-7 functions upstream of β -catenin in vertebrate dorsoventral mesoderm patterning.** *Development* 2000, 127:1981-1990.
The authors show that reducing Fzd7 function with antisense oligonucleotides in early *Xenopus* embryos results in defects in dorsal development. These data suggest that Fzd7 plays a critical role in the specification of dorsal cell fates and provides circumstantial evidence that a Wnt ligand is also required for this process.
84. Liu P, Wakamiya M, Shua MJ, Albrecht U, Behringer RR, Bradley A: **Requirement for Wnt3 in vertebrate axis formation.** *Nat Genet* 1999, 22:361-365.
This paper describes the expression pattern of Wnt3 in mice and the phenotype of embryos lacking Wnt3. The authors find that Wnt3⁺ mice develop a normal egg cylinder but do not form a primitive streak, mesoderm or node, indicating that Wnt3 plays an important role in axial patterning in the mouse.
85. Thorpe CJ, Schliesinger A, Bowerman B: **Wnt signalling in *Caenorhabditis elegans*: regulating repressors and polarizing the cytoskeleton.** *Trends Cell Biol* 2000, 10:10-17.
This review summarizes our knowledge of the role of Wnt signaling in regulating early cell fate decisions in the soil worm, *C. elegans*. The review discusses the interesting findings that in *C. elegans* Wnt signals regulate cell fate choices via two pathways: one that involves activation of a mitogen activated protein kinase and another that involves a heterotrimeric G-protein and polarization of the mitotic spindle.
86. Satoh S, Delgo Y, Furukawa Y, Kato T, Miwa N, Nishiwaki T, Kawasoe T, Ishiguro H, Fujita M, Toldno T, et al: **AXINI mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of AXINI.** *Nat Genet* 2000, 24:245-250.
This paper demonstrates that mutations in the AXINI gene are associated with hepatocellular carcinomas. These data indicate that Axin, like APC, is a tumor suppressor gene and strengthen the idea that genes involved in Wnt signaling play a critical role in human disease.
87. Nusse R, Varmus HE: **Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome.** *Cell* 1983, 31:99-109.
This paper examines the mechanism by which mouse mammary tumor virus causes mammary tumors and the authors find that proviral integration within the int1 (Wnt1) locus.
88. Nusse R, van Ooyen A, Cox D, Fung YK, Varmus H: **Mode of proviral activation of a putative mammary oncogene (int1) on mouse chromosome 15.** *Nature* 1984, 307:131-136.
This paper demonstrates that integration of mouse mammary tumor virus into the int1 (Wnt1) locus results in mis-expression of int1.
89. van Ooyen A, Nusse R: **Structure and nucleotide sequence of the putative mammary oncogene int-1; proviral insertions leave the protein-encoding domain intact.** *Cell* 1984, 39:233-240.
This paper presents the sequence of the int-1 (Wnt1) gene and describes the sites of proviral insertion in the 5' and 3' untranslated regions associated with tumorigenesis.
90. van Ooyen A, Kwee V, Nusse R: **The nucleotide sequence of the human int-1 mammary oncogene: evolutionary conservation of coding and non-coding sequences.** *EMBO J* 1985, 4:2905-2909.
This paper presents the sequence of the human int-1 (WNT1) gene and compares the sequence and organization of the human and mouse int-1 genes.
91. Arheden K, Mandahl N, Strombeck B, Isaksson M, Mielman F: **Chromosome localization of the human oncogene INT1 to**

<http://genomebiology.com/2001/3/1/reviews/3001.13>

- 12q13 by *in situ* hybridization. *Cytogenet Cell Genet* 1988, 47:86-87.
- This paper describes the chromosomal location of the human *INT1* (*WNT1*) gene to 12q13 a position involved in chromosomal rearrangements in lipomas, myxoid liposarcomas, pleomorphic adenomas, and myomas.
92. Walnwright BJ, Scambler PJ, Stanier P, Watson EK, Bell G, Wicking C, Estivill X, Courtney M, Boue A, Pedersen PS, et al: Isolation of a human gene with protein sequence similarity to human and murine *Int-1* and the *Drosophila* segment polarity mutant wingless. *EMBO J* 1988, 7:1743-1748.

This paper describes the cloning, sequence, and expression pattern of the human *WNT2* gene.

 93. McMahon JA, McMahon AP: Nucleotide sequence, chromosomal localization and developmental expression of the mouse *Int-1*-related gene. *Development* 1989, 107:643-650.

The authors describe the cloning, sequence, and expression pattern of the mouse *Wnt2* gene. Adult expression of mouse *Wnt2* is restricted to lungs and heart, and fetal expression, to the pericardium of the heart, to the umbilicus and associated allantoic mesoderm, and to the ventral lateral mesenchymal tissue surrounding the umbilical vein.

 94. Katoh M, Hirai M, Sugimura T, Terada M: Cloning, expression and chromosomal localization of *Wnt-13*, a novel member of the *Wnt* gene family. *Oncogene* 1996, 13:873-876.

This paper describes the cloning, sequence and mapping of the human *WNT13* gene to 1p13. The authors also show that *WNT13* is expressed in several cell lines including HeLa (cervical cancer), MKN28 and MKN74 (gastric cancer) cells.

 95. Grove EA, Tole S, Limon J, Yip L, Ragsdale CW: The hem of the embryonic cerebral cortex is defined by the expression of multiple *Wnt* genes and is compromised in *Gli3*-deficient mice. *Development* 1998, 125:2315-2325.

This paper describes the cloning of the mouse *Wnt2b* gene and expression analysis of *Wnt2b*, *Wnt3a*, *Wnt5a* and *Wnt7a* in the telencephalon. *Wnt2b*, *Wnt3a*, and *Wnt5a* were found to be expressed in the hem of the cerebral cortex and expression is absent in the *extra-toes1* mouse mutant.

 96. Zakin LD, Mazan S, Maury M, Martin N, Guenet JL, Brulet P: Structure and expression of *Wnt13*, a novel mouse *Wnt2* related gene. *Mech Dev* 1998, 73:107-116.

This paper describes the cloning and expression of the mouse *Wnt13* gene. *Wnt13* is expressed in the embryonic mesoderm during gastrulation and, at later stages, transcripts are detected in the dorsal midline of the diencephalon and mesencephalon, the heart primordia, the periphery of the lung bud and the otic and optic vesicles.

 97. Roelink H, Wagenaar E, Lopes da Silva S, Nusse R: *Wnt-3*, a gene activated by proviral insertion in mouse mammary tumors, is homologous to *Int-1/Wnt-1* and is normally expressed in mouse embryos and adult brain. *Proc Natl Acad Sci USA* 1990, 87:4519-4523.

The authors describe the characterization of the mouse *Wnt3* gene on chromosome 11 as a site for mouse mammary tumor pro-virus insertion. The paper also presents expression data for *Wnt3*.

 98. Chandrasekharappa SC, King SE, Freedman ML, Hayes ST, Bowcock AM, Collins FS: The CA repeat marker D17S791 is located within 40 kb of the *WNT3* gene on chromosome 17q. *Genomics* 1993, 18:728-729.

This paper describes the genomic localization of the human *WNT3* gene close to a CA repeat marker on chromosome 17q.

 99. Roelink H, Wang J, Black DM, Solomon E, Nusse R: Molecular cloning and chromosomal localization to 17q21 of the human *WNT3* gene. *Genomics* 1993, 17:790-792.

This paper describes the characterization of the human *WNT3* gene and its localization to chromosome 17q21. Analysis of the *WNT3* gene in a collection of mammary tumor samples failed to detect rearrangements or amplification.

 100. Huguet EL, McMahon JA, McMahon AP, Bicknell R, Harris AL: Differential expression of human *Wnt* genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue. *Cancer Res* 1994, 54:2615-2621.

The authors report the expression of *WNT3*, *WNT4*, and *WNT7b* in human breast cell lines and *WNT2*, *WNT3*, *WNT4*, and *WNT7b* in human breast tissues. *WNT3a* and *WNT7a* were not expressed in of the examined tissue. In addition, several of these genes, including *WNT2*, *WNT4* and *WNT7b*, showed increase expression in breast tumors.

 101. Roelink H, Nusse R: Expression of two members of the *Wnt* family during mouse development - restricted temporal and spatial patterns in the developing neural tube. *Genes Dev* 1991, 5:381-388.

This paper describes the cloning and expression patterns of the mouse *Wnt3* and *Wnt3a* genes. The authors compare and contrast the expression of the two genes in the developing nervous system and find that despite their high degree of sequence identity, *Wnt3* and *Wnt3a* are expressed in discrete regions of the spinal cord and brain.

 102. Greco TL, Takada S, Newhouse MM, McMahon JA, McMahon AP, Camper SA: Analysis of the vestigial tail mutation demonstrates that *Wnt-3a* gene dosage regulates mouse axial development. *Genes Dev* 1996, 10:313-324.

The authors present genetic and expression analyses demonstrating that the vestigial tail mutation is a hypomorphic allele of *Wnt3a*.

 103. Saitoh T, Hirai M, Katoh M: Molecular cloning and characterization of *WNT3A* and *WNT14* clustered in human chromosome 1q42 region. *Biochem Biophys Res Commun* 2001, 284:1168-1175.

This paper describes the sequence, expression, and mapping of the human *WNT3A* and *WNT14* genes. The genes were localized to chromosome 1q42 in a head to head manner separated by approximately 58 kb.

 104. Gavin BJ, McMahon JA, McMahon AP: Expression of multiple novel *Wnt-1/Int-1*-related genes during fetal and adult mouse development. *Genes Dev* 1990, 4:2319-2332.

The authors used a PCR-based strategy to isolate six *Wnt* genes (*Wnt4*, *Wnt5a*, *Wnt5b*, *Wnt6*, *Wnt7a* and *Wnt7b*) expressed in fetal mice.

 105. Clark CC, Cohen J, Eichstetter I, Cannizzaro LA, McPherson JD, Wasmuth JJ, Izozz RV: Molecular cloning of the human proto-oncogene *Wnt-5A* and mapping of the gene (*WNT5A*) to chromosome 3p14-p21. *Genomics* 1993, 18:249-260.

This paper describes the cloning and mapping of the human *WNT5A* gene. RT-PCR expression analysis of a variety of embryonic, neonatal, and adult cells and/or tissues showed that *WNT5A* was detected only in neonatal heart and lung.

 106. Adamson MC, Dennis C, Delaney S, Christiansen J, Monkley S, Kozak CA, Walnwright B: Isolation and genetic mapping of two novel members of the murine *Wnt* gene family, *Wnt11* and *Wnt12*, and the mapping of *Wnt5a* and *Wnt7a*. *Genomics* 1994, 24:9-13.

The authors cloned the mouse *Wnt11* and *Wnt12* (*Wnt10b*) genes by degenerate PCR and mapped both of these *Wnt* genes as well as the *Wnt5a* and *Wnt7a* genes. *Wnt11* mapped to chromosome 7, *Wnt12* (*Wnt10b*) to chromosome 15 close to *Wnt1*, *Wnt5a* to chromosome 14, and *Wnt7a* to chromosome 6.

 107. Saitoh T, Katoh M: Molecular cloning and characterization of human *WNT5B* on chromosome 12p13.3 region. *Int J Oncol* 2001, 19:347-351.

Expression analysis shows that *WNT5A* is expressed in adult prostate and fetal brain, and weakly expressed in fetal lung, kidney, adult liver, ovary, and small intestine. *WNT5B* is also expressed in the gastric cancer cell lines MKN7, MKN45, KATO-III, and a laryngeal carcinoma cell line NT2.

 108. Rankin J, Sprachan T, Lako M, Lindsay S: Partial cloning and assignment of *WNT6* to human chromosome band 2q35 by *in situ* hybridization. *Cytogenet Cell Genet* 1999, 84:50-52.

This paper reports the cloning of a partial cDNA encoding *WNT6* and its mapping to chromosome 2q35.

 109. Kirikoshi H, Sekihara H, Katoh M: *WNT10A* and *WNT6*, clustered in human chromosome 2q35 region with head-to-tail manner, are strongly coexpressed in SW480 cells. *Biochem Biophys Res Commun* 2001, 283:798-805.

This paper describes the cloning and mapping of the human *WNT6* and *WNT10A* genes. The two genes are clustered in the 2q35 region separated by only 7 kb. Both genes are expressed in a variety of tissues, including kidney, placenta, and spleen, and cancer cell lines.

 110. Ikegawa S, Kumano Y, Okui K, Fujiwara T, Takahashi E, Nakamura Y: Isolation, characterization and chromosomal assignment of the human *WNT7A* gene. *Cytogenet Cell Genet* 1996, 74:149-152.

This paper describes the characterization of the human *WNT7A* gene and its expression in placenta, kidney, testis, uterus, fetal lung, and fetal and adult brain.

 111. Bui TD, Lako M, Lejeune S, Curtis AR, Sprachan T, Lindsay S, Harris AL: Isolation of a full-length human *WNT7A* gene implicated in limb development and cell transformation, and mapping to chromosome 3p25. *Gene* 1997, 189:25-29.

This paper describes the cloning and mapping of human *WNT7A*.

112. van Bokhoven H, Kissing J, Schapens M, van Beersum S, Simons A, Riegman P, McMahon JA, McMahon AP, Brunner HG: Assignment of WNT7B to human chromosome band 22q13 by *in situ* hybridization. *Cytogenet Cell Genet* 1997, 77:286-289.
This paper describes the mapping of human WNT7B to chromosome 22q13.
113. Kirikoshi H, Sekihara H, Katoh M: Molecular cloning and characterization of human WNT7B. *Int J Oncol* 2001, 19:779-783.
The authors describe the characterization of the human WNT7B gene and its expression in fetal brain, lung and kidney, and in adult brain, lung and prostate. WNT7B is also expressed in a lung cancer cell line A549, esophageal cancer cell lines TE2, TE3, TE4, TE5, TE6, TE7, TE10, TE12, a gastric cancer cell line TMK1, and pancreatic cancer cell lines BxPC-3, AsPC-1 and Hs766T. In addition, WNT7B was found to be up regulated in 50% of primary gastric cancers.
114. Bouillet P, Oulad-Abdelghani M, Ward SJ, Bronner S, Chambon P, Dolle P: A new mouse member of the Wnt gene family, mWnt-8, is expressed during early embryogenesis and is ectopically induced by retinoic acid. *Mech Dev* 1996, 58:141-152.
The authors describe the cloning of the mouse Wnt8 gene and its expression during embryogenesis. Wnt8 is expressed in the posterior region of the epiblast of early primitive streak-stage embryos and as gastrulation proceeds expression spreads into the embryonic ectoderm. Wnt8 is also transiently expressed in the mesoderm.
115. Saitoh T, Katoh M: Molecular cloning and characterization of human WNT8A. *Int J Oncol* 2001, 19:123-127.
This paper presents the sequence and organization of the human WNT8A gene. Expression analysis of WNT8A in various human tissues and cell lines only detected transcripts in NT2 teratocarcinoma cells.
116. Lako M, Strachan T, Curris AR, Lindsay S: Isolation and characterization of WNT8B, a novel human Wnt gene that maps to 10q24. *Genomics* 1996, 35:386-388.
This paper and [117] describe the cloning, mapping, and expression analysis of the human WNT8B gene and [117] presents expression data for the mouse Wnt8b gene. Both the human and mouse Wnt8b genes were restricted to the developing brain, with the majority of expression being found in the forebrain.
117. Lako M, Lindsay S, Bullen P, Wilson DI, Robson SC, Strachan T: A novel mammalian wnt gene, WNT8B, shows brain-restricted expression in early development, with sharply delimited expression boundaries in the developing forebrain. *Hum Mol Genet* 1998, 7:813-822.
See [116].
118. Richardson M, Redmond D, Watson CJ, Mason JC: Mouse Wnt8b is expressed in the developing forebrain and maps to chromosome 19. *Mamm Genome* 1999, 10:923-925.
The authors present the mapping of mouse Wnt8b and characterize its expression in the developing forebrain. See also [117].
119. Wang J, Shackleford GM: Murine Wnt10a and Wnt10b: cloning and expression in developing limbs, face and skin of embryos and in adults. *Oncogene* 1996, 13:1537-1544.
The authors report the isolation of the mouse Wnt10a and Wnt10b genes as well as analyses of the expression patterns of these genes in adult and embryonic tissues. In adults, Wnt10a RNA was most abundant in adult brain with a high concentration in the pituitary gland. Wnt10b was highest in lung and uterus, and mRNAs of both genes were detected in thymus and spleen. In embryos, expression was found in a variety of tissues including limbs, face, skin, and liver.
120. Christensen JH, Dennis CL, Wicking CA, Monkley SJ, Willkinson DG, Wainwright BJ: Murine Wnt-11 and Wnt-12 have temporally and spatially restricted expression patterns during embryonic development. *Mech Dev* 1995, 51:341-350.
This paper describes the expression patterns of mouse Wnt11 and Wnt12 (Wnt10b). Wnt11 expression is first detected within the truncus arteriosus and is later detected in the somites at the junction of the dermatome and the myotome and in limb bud mesenchyme. Wnt12 (Wnt10b) is also expressed in the limb and is expressed in the apical ectodermal ridge.
121. Lee FS, Lano TF, Kuo A, Shackleford GM, Leder P: Insertional mutagenesis identifies a member of the Wnt gene family as a candidate oncogene in the mammary epithelium of int-2/Fgf-3 transgenic mice. *Proc Natl Acad Sci USA* 1995, 92:2268-2272.
This paper presents the characterization of the mouse Wnt10b gene as a site of mouse mammary tumor virus insertion in int-2/Fgf-3 transgenic mice that cooperate with int-2/Fgf-3 in tumorigenesis. The authors also showed that Wnt10b is expressed in the embryo and mammary gland of virgin but not pregnant mice. See also [123].
122. Bui TD, Rankin J, Smith K, Huguet EL, Ruben S, Strachan T, Harris AL, Lindsay S: A novel human Wnt gene, WNT10B, maps to 12q13 and is expressed in human breast carcinomas. *Oncogene* 1997, 14:1249-1253.
This paper describes the characterization of the human WNT10B gene showing that it maps to 12q13 in close proximity to WNT1. The authors also examine the expression of WNT10b in human breast cancers. See also [124].
123. Hardiman G, Albright S, Tsunoda J, McClanahan T, Lee F: The mouse Wnt-10B gene isolated from helper T cells is widely expressed and a possible oncogene in BR6 mouse mammary tumorigenesis. *Gene* 1996, 172:199-205.
This paper describes the cloning of mouse Wnt10b and its expression in embryos and adults. In addition, the Wnt10b gene is shown to be an insertion site for mouse mammary tumor virus and may contribute to mammary tumors in BR6 mice. See also [121].
124. Hardiman G, Kastalian RA, Bazzan JF: Isolation, characterization and chromosomal localization of human WNT10B. *Cytogenet Cell Genet* 1997, 77:278-282.
This paper presents the cloning and mapping of the human WNT10B gene to 12q13. The expression pattern of WNT10B reveals that it is present in many adult tissues, with the highest levels found in heart and skeletal muscle, and is also expressed in several human cancer cell lines, including HeLa cells. See also [122].
125. Lako M, Strachan T, Bullen P, Wilson DI, Robson SC, Lindsay S: Isolation, characterization and embryonic expression of WNT11, a gene which maps to 11q13.5 and has possible roles in the development of skeleton, kidney and lung. *Gene* 1998, 219:101-110.
The authors characterize the human WNT11 gene, mapping it to 11q13.5 and demonstrating its expression in the perichordium of the developing skeleton, lung mesenchyme, the tips of the ureteric buds and other areas of the urogenital system and the cortex of the adrenal gland.
126. Bergstein I, Eisenberg LM, Bhalerao J, Jenkins NA, Copeland NG, Osborne MP, Bowcock AM, Brown AM: Isolation of two novel WNT genes, WNT14 and WNT15, one of which (WNT15) is closely linked to WNT3 on human chromosome 17q21. *Genomics* 1997, 46:450-458.
This paper describes the cloning and mapping of human WNT14 and WNT15 and show that WNT13 (WNT7B) is expressed in mammary tissue.
127. McWhirter JR, Neuteboom ST, Wanciewicz EY, Monia BP, Downing JR, Murte C: Oncogenic homeodomain transcription factor E2A-Pbx1 activates a novel WNT gene in pre-B acute lymphoblastic leukemia. *Proc Natl Acad Sci USA* 1999, 96:11464-11469.
The authors characterize the human WNT16 gene and show that it is activated by the E2A-Pbx1 fusion protein in pre-B acute lymphoblastic leukemia. WNT16 is normally expressed in the spleen, appendix, and lymph nodes, but not in bone marrow. However, WNT16 transcripts are highly expressed in bone marrow and cell lines derived from pre-B ALL patients carrying the E2A-Pbx1 fusion suggesting that inappropriate expression of WNT16 plays a role in leukemia.
128. Fear MW, Kelsell DP, Spurr NK, Barnes MR: Wnt-16a, a novel Wnt-16 isoform, which shows differential expression in adult human tissues. *Biochem Biophys Res Commun* 2000, 278:814-820.
The authors map human WNT16 to 7q31 and characterize the differential expression of two distinct WNT16 isoforms. The isoforms were shown to utilize different 5'-UTRs and first exons.
129. McMahon AP, Gavin BJ, Parr B, Bradley A, McMahon JA: The Wnt family of cell signalling molecules in postimplantation development of the mouse. *Ciba Found Symp* 1992, 168:199-212.
This paper summarizes the phenotype of the Wnt1 knockout mice. See [74].
130. Mastick GS, Fan CM, Tessier-Lavigne M, Serbedzija GN, McMahon AP, Easter SS, Jr: Early deletion of neuromeres in Wnt-1^{-/-} mutant mice: evaluation by morphological and molecular markers. *J Comp Neurol* 1996, 374:246-258.
This paper builds on [74] and provides a detailed characterization of the phenotype of Wnt1 deficient mice focusing on possible perturbations in structures adjacent to the presumptive midbrain and rhombellum.
131. Ikaya M, Lee SM, Johnson JE, McMahon AP, Takada S: Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* 1997, 389:966-970.
The authors show that mice mutant for both Wnt1 and Wnt3 show a dramatic decrease in the number of neural crest progenitors, normally derived from the dorsal neural tube.

<http://genomebiology.com/2001/3/1/reviews/3001.15>

132. Monkley SJ, Delaney SJ, Pennisi DJ, Christiansen JH, Wainwright BJ: Targeted disruption of the *Wnt2* gene results in placenta-tion defects. *Development* 1996, 122:3343-3353.
This paper examines the phenotype of *Wnt2* knockout mice and show that mice lacking *Wnt2* display runting and approximately 50% died perinatally. Mutant mice were found to have defects in the size and structure the placenta with notable perturbation of the vascularization of the placenta.
133. Millar SE, Willert K, Salinas PC, Roelink H, Nusse R, Sussman DJ, Barish GS: WNT signaling in the control of hair growth and structure. *Dev Biol* 1999, 207:133-149.
This paper shows that overexpression of *Wnt3* in skin of transgenic mice results in a short hair phenotype implicating Wnt signaling in hair growth. Overexpression of Dishevelled-2 (*Dvl2*) in outer root sheath cells mimicked this phenotype.
134. Kishimoto J, Burgeson RE, Morgan BA: Wnt signaling maintains the hair-inducing activity of the dermal papilla. *Genes Dev* 2000, 14:1181-1185.
The authors show that specific *Wnt* genes can maintain anagen-phase gene expression in isolated dermal papilla cells in vitro and hair induc-tive activity in a skin reconstitution assay.
135. Takada S, Stark KL, Shea MJ, Vasileva G, McMahon JA, McMahon AP: Wnt-3a regulates somite and tailbud formation in the mouse embryo. *Genes Dev* 1994, 8:174-189.
This paper and [136] describe the phenotype of mice lacking the *Wnt3a* gene. *Wnt3a*^{-/-} embryos lack caudal somites, have a disrupted noto-chord, and fail to form a tailbud. Mutant mice also possess an ectopic neural tube suggesting that *Wnt3a* plays a critical role in specifying paraxial mesoderm and that in its absence those cells adopt neural fates.
136. Yoshikawa Y, Fujimori T, McMahon AP, Takada S: Evidence that absence of *Wnt-3a* signaling promotes neuralization instead of paraxial mesoderm development in the mouse. *Dev Biol* 1997, 183:234-242.
See [135].
137. Yamaguchi TP, Takada S, Yoshikawa Y, Wu N, McMahon AP: T (*Brachyury*) is a direct target of *Wnt3a* during paraxial mesoderm specification. *Genes Dev* 1999, 13:3185-3190.
This paper shows that the T-box gene, *brachyury*, is down regulated in mice lacking *Wnt3a*. Transgenic analysis of the *brachyury* promoter further demonstrates that *brachyury* is a direct target of the Wnt pathway acting downstream of *Wnt3a*.
138. Lee SM, Tolia S, Grove E, McMahon AP: A local *Wnt-3a* signal is required for development of the mammalian hippocampus. *Development* 2000, 127:457-467.
The authors examine the role of *Wnt3a* in the developing brain and show that in mice lacking *Wnt3a*, caudomedial progenitor cells in the cerebral cortex underproliferate. By mid-gestation, this defect leads to the absence of the hippocampus or very small populations of residual hippocampal cells.
139. Yamaguchi TP, Bradley A, McMahon AP, Jones S: A *Wnt5a* pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* 1999, 126:1211-1223.
The authors characterize the phenotype of *Wnt5a* knockout mice showing that *Wnt5a* is required for appropriate growth of a variety of tissues including the anterior-posterior axis, limbs, and developing face, ears and genitals.
140. Parr BA, Avery EJ, Cygan JA, McMahon AP: The classical mouse mutant postaxial hemimelia results from a mutation in the *Wnt7a* gene. *Dev Biol* 1998, 202:228-234.
This paper examines the molecular defect underlying the postaxial hemimelia (*px*) mutant and show by morphological analysis and breed-ing experiments that the *px* phenotype is caused by a mutation in the *Wnt7a* gene. Molecular analysis demonstrates that *px* mice harbor a 515-bp deletion in the *Wnt7a* gene that results in the production of a truncated *Wnt7a* protein.
141. Miller C, Sassoon DA: *Wnt-7a* maintains appropriate uterine patterning during the development of the mouse female reproductive tract. *Development* 1998, 125:3201-3211.
This paper examines the defects associated with loss of the *Wnt7a* gene in female mice. The authors demonstrate that *Wnt7a* is required for appropriate patterning of the oviduct and uterus as well as disorgani-zation of the uterine smooth muscle.
142. Parr BA, Cornish VA, Cybulsky MI, McMahon AP: *Wnt7b* regulates placental development in mice. *Dev Biol* 2001, 237:324-332.
This paper shows that targeted disruption of the mouse *Wnt7b* gene results in placental defects including inhibition of the normal fusion of the chorion and allantois, perhaps due to the loss of integrin alpha-4.
143. Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA: Inhibition of adipogenesis by Wnt signaling. *Science* 2000, 289:950-953.
The authors show that Wnt signaling maintains preadipocytes in an undifferentiated state through inhibition of adipogenic-promoting tran-scription factors.

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☒ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.